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Pathway risk analysis of weed seeds in imported grain: A Canadian perspective

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Abstract

The risk of introducing weeds to new areas through grain (cereals, oilseeds and pulses) intended for processing or consumption is typically considered less than that from seed or plants for planting. However, within the range of end uses for grain, weed risk varies significantly and should not be ignored. In this paper, we discuss pathway risk analysis as a framework to examine the association of weed seeds with grain commodities throughout the production process from field to final end use, and present inspection sampling data for grain crops commonly imported to Canada. In the field, weed seed contamination of grain crops is affected by factors such as country of origin, climate, biogeography and production and harvesting practices. As it moves toward export, grain is typically cleaned at a series of elevators and the effectiveness and degree of cleaning are influenced by grain size, shape and density as well as by grade requirements. In cases where different grain lots are blended, uncertainty may be introduced with respect to the species and numbers of weed seed contaminants. During transport and storage, accidental spills and cross-contamination among conveyances may occur. At the point of import to Canada, inspection sampling data show that grain shipments contain a variety of contaminants including seeds of regulated weeds and species that represent new introductions. However, grain cleaning and processing methods tailored to end use at destination also affect the presence and viability of weed seeds. For example, grains that are milled or crushed for human use present a lower risk of introducing weed seeds to new environments than grains that undergo minimal or no processing for livestock feed, or screenings that are produced as a by-product of grain cleaning. Pathway risk analysis allows each of these stages to be evaluated in order to characterize the overall risk of introducing weeds with particular commodities, and guide regulatory decisions about trade and plant health.

Keywords

Pathway risk analysis, pest risk assessment, weed seeds, contaminants, grain, imports, screenings, Canada

Introduction

Internationally traded grain commodities are recognized as a pathway for the introduction of weed seeds into new areas (Hodkinson and Thompson 1997; Benvenuti 2007; Shimono and Konuma 2008; Shimono et al. 2015). Grain is defined as "seeds intended for processing or consumption and not for planting" (IPPC 2015) and grain commodities typically consist of bulk shipments of cereal, oilseed or pulse crops destined for use as human food, livestock feed or industrial products. Many weed seeds associated with grain crops in the field are harvested along with the crop and can be difficult to remove due to similarities in shape and size of the seeds (Benvenuti 2007; Michael et al. 2010; Salisbury and Frick 2010). Depending on the destination and intended end use of the grain some of these seeds may be introduced into new environments suitable for growth and establishment. Because large volumes of grain are traded internationally each year, this pathway may represent a considerable contribution to the spread of new agricultural pests around the world. Several studies have reported large numbers of contaminant weed species found in sampled grain commodities (Pheloung et al. 1999a; Kurokawa 2001; Shimono and Konuma 2008; Darbyshire and Allison 2009; Mekky 2010) and a number of globally important weeds of agriculture are thought to have been spread as contaminants in grain (e.g., Jehlík and Hejný 1974; Jehlík and Dostálek 2008).

Regulating the spread of weeds via this pathway is the responsibility of individual countries under the guidelines of the International Plant Protection Convention (IPPC), and many countries have legislation and import requirements that mitigate the risk of introducing new weed species to some degree. However, according to the principles of the IPPC, regulations must be based on risk analysis and characterizing the risk associated with complex pathways such as this one remains a challenge. International standards for pest risk analysis are well developed for addressing individual species in terms of the likelihood they will enter, establish and spread in a new area, and the impacts they may have (IPPC 2007; 2013). Likewise, a number of weed risk assessment methods have been published and evaluated for their ability to separate weeds from non-weeds and predict which plant species are likely to be most invasive (e.g., Pheloung et al. 1999b; Daehler et al. 2004; Gordon et al. 2008; McClay et al. 2010; Koop et al. 2011). Such approaches are used by countries around the world to guide the development of plant health regulations, and are helpful for identifying and preventing the introduction of particular pests of concern; however, they are less well developed for addressing the risk associated with pathways. A species-by-species approach is often impractical for commodities like grain where a single shipment could be harbouring hundreds of different weed contaminants.

More recently, a pathways approach to pest risk analysis has been proposed (NAPPO 2012), which shifts the focus onto the characteristics of the pathway itself and events along the pathway that may have significance for pest prevalence or pest risk. In this context, a pathway is defined as "any means that allows the entry or spread of a pest" (IPPC 2015), and risk is characterized in terms of events

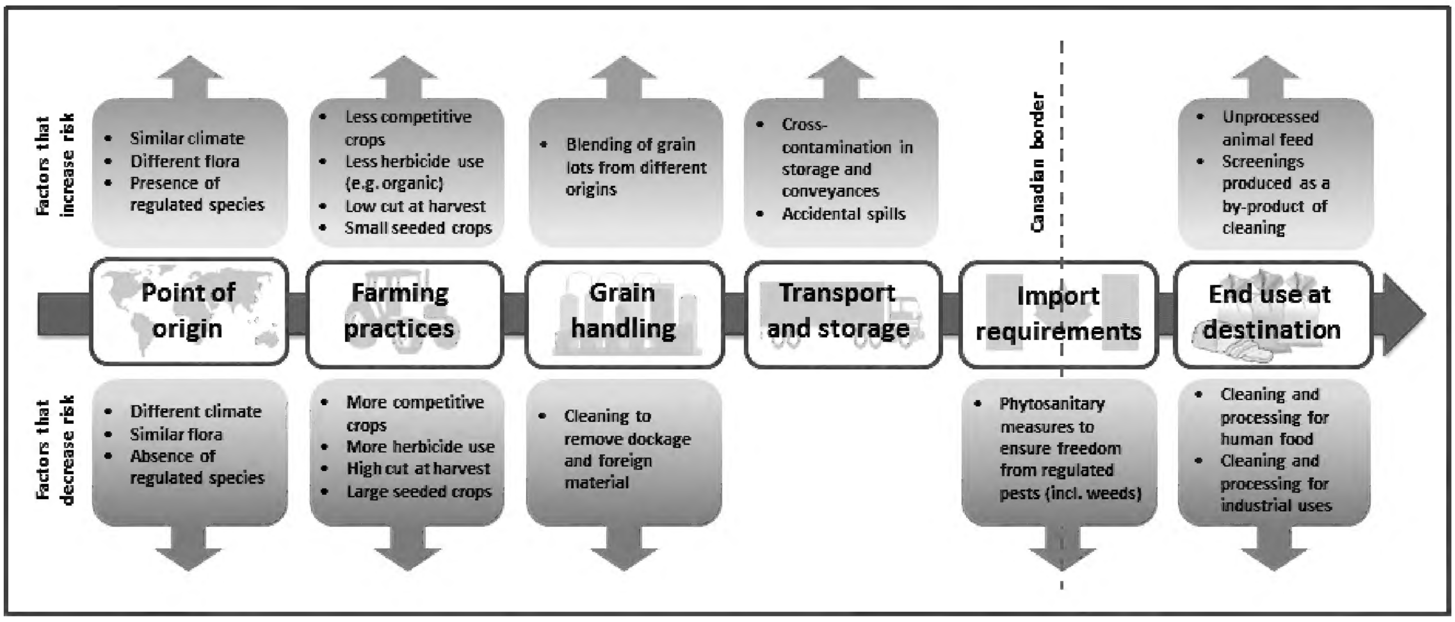

Figure 1. Conceptual diagram of imported grain as a pathway for the introduction of weed seeds. Six points, or events, along the pathway that have relevance for weed risk are illustrated from left to right along a timeline from point of origin to end use at destination. Factors that increase the risk of introducing new weed species to Canada are shown in red boxes, while factors that decrease the risk are shown in green boxes.

that affect the whole suite of pests associated with the pathway, without requiring a species-by-species focus. To do this, the pathway must first be described, and the individual events of interest identified. Each "event" can then be examined in terms of its implications for pest risk and whether it offers potential for risk mitigation. Although pest risk inherently depends on both the likelihood and consequences of pest introduction, the focus of a pathway risk analysis is often on the likelihood of pest introduction and/or spread, with consequences assumed or understood from prior studies (NAPPO 2012).

In this paper we discuss the association of weed seeds with imported grain from point of origin to end use at destination, and provide a qualitative description of the pathway that can be used as a framework for pathway risk analysis. We identify six points, or events, along the pathway that have relevance for weed risk, namely: crop-weed associations at the point of origin; farming practices; grain handling practices; transport and storage; import requirements; and end use of grain in the country of destination (Canada) (Figure 1). We discuss each of these in further detail below. We also present inspection sampling data for weeds in ten grain crops most commonly imported to Canada: corn, rice, soybean, cereals, pulses, canola or rapeseed, sunflower, flax, millet and sorghum. Canada imports about 2 million metric tonnes of these crops combined each year (FAO 2015; Statistics Canada 2016) (Table 1).

Crop-weed associations at the point of origin

The pathway for weed seed dispersal in grain begins in the field where the crop is grown in the country of origin. The majority of Canadian grain is imported from

Table 1. Canadian grain imports 2010–2015. Average annual imports in MT/yr for ten grain crops or crop groupings most commonly imported to Canada, and top five countries of origin for each crop given as % total imports over the period 2010–2015. Note that import data includes seed for planting in addition to grain but this typically makes up a very small proportion (<5%) of the total for each crop (data from: Statistics Canada 2016).

Crop	Scientific name	MT/yr	Top five countries of origin (% total imports)				
Corn	*Zea mays* L. subsp. *mays*	1,065,056	United States (97.5%)	India (0.8%)	Romania (0.5%)	Russian Fed. (0.4%)	Chile (0.2%)
Rice	*Oryza sativa* L.	385,065	United States (60.7%)	Thailand (22.0%)	India (9.6%)	Pakistan (2.5%)	Italy (1.2%)
Soybean	*Glycine max* (L.) Merr.	282,962	United States (85.0%)	India (10.4%)	China (1.9%)	Ukraine (0.7%)	Turkey (0.7%)
Cereals Wheat Oats Barley	 *Triticum aestivum* L. *Avena sativa* L. *Hordeum vulgare* L. subsp. *vulgare*	142,315	United States (76.6%)	Denmark (11.7%)	Romania (4.6%)	Sweden (1.4%)	Rep. of Ireland (1.3%)
Pulses Beans Peas Chickpeas Lentils	 *Phaseolus* spp., *Vigna* spp., *Vicia* spp. *Pisum sativum* L. *Cicer arietinum* L. *Lens culinaris* Medik.	125,691	United States (75.6%)	Australia (4.8%)	China (4.1%)	Thailand (2.8%)	India (2.2%)
Canola	*Brassica napus* L., *B. rapa* L., *B. juncea* (L.) Czern.	116,781	United States (94.7%)	Chile (5.0%)	Australia (0.1%)	Ukraine (0.1%)	Uruguay (0.01%)
Sunflower	*Helianthus annuus* L.	28,495	United States (86.6%)	Bulgaria (5.5%)	China (3.4%)	Argentina (2.9%)	Italy (0.5%)
Flax	*Linum usitatissimum* L.	10,575	United States (74.7%)	Argentina (7.1%)	Russian Fed. (5.5%)	Kazakhstan (2.8%)	India (2.7%)
Millet Proso millet Foxtail millet Japanese millet Pearl millet	 *Panicum miliaceum* L. subsp. *miliaceum* *Setaria italica* (L.) P. Beauv. subsp. *italica* *Echinochloa frumentacea* Link, *E. esculenta* (A. Braun) H. Scholz *Pennisetum glaucum* (L.) R. Br.	10,144	United States (82.7%)	Ukraine (6.3%)	China (3.1%)	Russian Fed. (2.3%)	India (1.8%)
Sorghum	*Sorghum bicolor* (L.) Moench subsp. *bicolor*	5,114	United States (93.8%)	India (2.5%)	Argentina (1.5%)	Ethiopia (1.3%)	China (0.5%)
Total (all crops)		2,172,198					

the U.S., although significant amounts are also brought in from other countries, and trade patterns frequently shift to meet market demands (FAO 2015; Statistics Canada 2016) (Table 1). On a broad scale, weed communities and species assemblages are determined by geography and vary according to the crop species and conditions (e.g., climate, soils) in the country or area of origin. This information is usually available from import documentation and using this, along with national or regional floras, agricultural statistics and published literature on weed-crop associations, an initial analysis can be made of the weed communities and species expected to be associated with the crop in the field. For example, most corn imported to Canada originates in the U.S. Data from the U.S. Department of Agriculture shows that corn is harvested for grain in 41 states, with the majority coming from the Midwest, from states such as Iowa, Illinois, Nebraska, Minnesota and Indiana (USDA-NASS 2010, 2012). A large amount of information exists on weeds of the U.S. corn belt, and lists of common species are readily available (e.g., velvetleaf (*Abutilon theophrasti* Medik.), lamb's-quarters (*Chenopodium album* L.), wild proso millet (*Panicum miliaceum* L.), woolly cupgrass (*Eriochloa villosa* (Thunb.) Kunth), foxtails (*Setaria* spp.), and pigweeds (*Amaranthus* spp.)) (Forcella et al. 1992; Forcella et al. 1996; Forcella et al. 1997; Myers et al. 2004; Clay et al. 2005; Davis et al. 2005; Gibson et al. 2005; Davis 2008). Although the exact species and numbers of weeds present will vary from field to field and season to season in response to local conditions, farming practices and weather, it is possible to use this type of information to develop a preliminary picture of the weeds likely to be associated with the crop at the point of origin.

The risk of introducing new weed species to Canada depends not only on the number of weed seeds contaminating imported grain, but on the particular species assemblages present, and the likelihood they will end up in suitable environments for establishment and spread. Many contaminants moving in the international grain trade may be common weeds already present in Canada, and thus do not present a risk of new species introductions. Others may be weeds from tropical climates unlikely to survive through Canadian winters, or weeds associated with crops not widely grown in Canada (e.g., rice). At a broad scale, information about the point of origin allows for generalizations about risk. For example, the risk of new species introductions is generally considered lower from countries with similar weed floras (i.e., fewer new species) or different climates (i.e., species less likely to survive), and higher from countries with different weed floras and similar climates. At this stage there is also the opportunity to determine whether particular weed species of concern (e.g., regulated species) occur in the area of origin. The level of risk will vary for each crop/country combination proposed for importation, and the more detailed the information about point of origin (e.g., state, county), the more specific the analysis can be. However, it should be noted that the value of a very detailed analysis at this stage may be compromised by industry practices further along the pathway, for example blending of grain lots from different origins (see Grain handling, below).

Farming practices

At smaller scales, crop production practices can also impact the diversity and prevalence of weeds at the field level and at harvest.

Crop production: Prior to planting, factors such as previous land use, crop rotation, pre-planting tillage, herbicide application, seed bank composition and crop seed purity can play a role in characterizing a field's weed flora for a particular year (Thomas and Dale 1991; Blackshaw et al. 2006). At planting time, grower decisions about crop type, planting date and planting density will influence the crop's ability to compete with weeds (Swanton and Weise 1991). Throughout the growing season, climatic factors, fertilization and weed control decisions can further affect the performance of both weeds and crops. In general, weeds with similar biology and requirements to those of crops tend to be favoured (Thomas and Dale 1991), with well-known examples including jointed goatgrass (*Aegilops cylindrica* Host) in wheat, Johnson grass (*Sorghum halepense* (L.) Pers.) in sorghum, and wild mustard (*Sinapis arvensis* L.) in canola.

Some crops and crop cultivars are inherently more competitive than others. Crop competitive ability varies from region to region, but a general ranking puts cereals first, followed by canola and then pulses (Blackshaw et al. 2002). Highly competitive crops are able to germinate, emerge and accumulate biomass more rapidly than weeds and have an advantageous height and canopy structure for intercepting light (AAFC 2015). Winter annual crops generally have an advantage over spring-seeded crops in that they overwinter as seedlings and are poised for rapid growth in spring (AAFC 2015). Conversely, with the exception of field pea, pulse crops are generally poor competitors against weeds due to slow initial growth, short stature and inability to quickly close the crop canopy (Saskatchewan Pulse Growers 2000; Pulse Crop Work Group 2002; McKay et al. 2003; Corp et al. 2004).

Chemical weed control options also vary by crop. In general, broadleaved weeds are easier to control in cereals and other monocot crops, while grass weeds are easier to control in broadleaved crops. For some crops, such as flax and pulses, herbicide options tend to be more limited than those for others, such as cereal grains or corn (OMAFRA 2009). Herbicide tolerant cultivars of crops such as corn, soybean and canola allow more comprehensive weed control than many conventional varieties, reducing the number of weeds in the field (Shaw and Bray 2003; O'Donovan et al. 2006) and changing the species composition of weed communities (Webster and Nichols 2012). On the other hand, the rise of herbicide resistant weeds may reduce the advantages of herbicide tolerant cultivars over time, as herbicide resistant weed seeds are dispersed as seed and grain contaminants around the globe (Shimono et al. 2010).

In the case of organically grown crops, a variety of non-chemical weed control options, such as mechanical and thermal methods, mulching and intercropping, may be employed to keep weeds in check (Bond and Grundy 2001). Floral diversity is promoted and the presence of some weeds at acceptable levels may be beneficial to the system in terms of nutrient cycling and pest and disease control (Stockdale et al. 2001). As a result, the quantity and composition of weed seeds in organic grain can

differ significantly from that which is conventionally grown (e.g., Marshall et al. 2003; Bengtsson et al. 2005).

Harvest: At harvest, critical factors contributing to weed contamination levels include timing, weather conditions, crop vs. weed height, weed maturity and combine settings (Forcella et al. 1996; Davis 2008; Shimono and Konuma 2008). Grain crops are usually harvested by direct combining or a sequence of swathing then combining, and weeds most likely to be harvested with the crop are those that are taller than cutting height at the time of harvest, with mature seed retained in the seed heads. Early maturing weed species shed most or all of their seeds prior to harvest, though some seed may be retained during cool years (e.g., *Sinapis arvensis* in corn, Forcella et al. 1996). Volunteer crops can also be problematic at harvest, as they are usually resistant to shattering (Shimono and Konuma 2008). In taller crops, seeds from short species are generally eliminated during harvesting (Shimono and Konuma 2008). For example, sunflower is one of the cleanest grains taken into a mill when the combine is set high at harvest (Pierce 1970). On the other hand, pulse crops are low-growing and harvested close to the ground, making them more likely to be contaminated with weed seeds. In crops that are swathed prior to combining (e.g., canola), weeds of any height with mature seeds attached may be subsequently harvested with the swaths.

The action of the conventional combine includes reaping, threshing (separating the grain from the husks) and winnowing (blowing off fines and other foreign material). Weed seeds that have a pappus are easily dislodged and dispersed at harvest time and are more readily eliminated during the cleaning process (Shimono and Konuma 2008). The amount of weed seeds in grain can be reduced at harvest with correct combine sieve and fan adjustment (Humburg et al. 2009). This tends to be easier with large-seeded crops like corn and soybean than for smaller-seeded crops like cereals, canola, flax and millet. For example, in a two year study of timing and measurement of weed seed shed in four corn plots from west central Minnesota, it was observed that harvested corn grain samples were free of weeds, indicating that most had been dispersed by the harvesting machinery (Forcella et al. 1996). In contrast, a study of the effect of harvesting and cleaning on weed seed contamination in wheat reported a significant level of contamination (Shimono and Konuma 2008).

Overall, knowledge of crop production and harvesting practices can be helpful for considering their effect on grain contamination at source. Although weed levels and species complexes vary from farm to farm, with different agronomic, harvesting and cleaning practices, generalizations can be made based on the information available and applied to the evaluation of risk. For example, crops that are typically more competitive, treated with herbicides, harvested at a greater height or have large seeds might be expected to harbour less weed seed contaminants (lower risk) than crops that are less competitive, grown organically, harvested close to the ground, or that have small seeds that are difficult to separate from weed seeds (higher risk). This information can be combined with that collected about point of origin to develop a more refined picture of the species and levels of weed contamination that might be expected with a particular grain crop after harvest.

Grain handling

From the farm, harvested grain typically moves through a series of elevators on its way to export, where it is cleaned and graded to determine its market value.

Cleaning: Cleaning removes dockage, which is material that can readily be removed from grain prior to grading, such as stones, straw, chaff, broken grains, contaminant seeds, dust and hulls (CGC 2015). It may be done on-farm, at local, sub-terminal or export elevators, or when grain is received at feed mills or processing plants (Lin and Lin 1994; Lin 1996; Wilson et al. 2000; U.S. Soybean Export Council 2008). Conventional seed cleaning includes the use of aspirators, screens, gravity tables and other separators to remove debris and weed seeds from the crop based on size, shape or weight. As with harvesting, larger-seeded crops (e.g., corn, soybean) are relatively easier to clean than smaller-seeded crops (e.g., flax, millet), as there tends to be less overlap with weed seeds in terms of seed dimensions and weight (Salisbury and Frick 2010).

Grading: The extent to which grain is cleaned is typically determined by grade requirements to meet government regulations, export standards or contract conditions. Numerical grades are a measure of grain quality and cleanliness and help determine the value of grain on the market (Lin and Lin 1994; Lin 1996; U.S. Soybean Export Council 2008; USDA-FGIS 2015). Allowances for quality (e.g., minimum test weight, heat damaged kernels) and cleanliness (e.g., percent foreign material) are specified for each grade of a given crop, with the highest grade representing the highest quality. Weed seeds form a component of the dockage or foreign material (FM) of the grain. In some cases, maximum limits by grade of certain weed species are also stipulated (USDA-FGIS 2015).

The percentage of FM allowed in a grade can be an indicator of the level of contamination with weed seeds. For example, U.S. No. 1 grade soybeans must contain no more than 1% FM by weight, U.S. No. 2 grade no more than 2%, U.S. No. 3 grade no more than 3%, and so on (USDA-FGIS 2015). Using import data by grade, it is possible to estimate the maximum amount of FM that might be imported along with the crop. It is important to note that this represents a maximum, and some imports may have contamination levels below the allowable limits. In addition, FM consists of more than just weed seeds and the proportion may vary from crop to crop and shipment to shipment (Bell and Shires 1980; Hill et al. 1994; Lin and Lin 1994; Lin 1996). However, it is a useful indicator of scale; for example, for grain commodities that are imported in the range of 100,000 MT (e.g., cereals, pulses) - 1 million MT (e.g., corn) per year, 1% would represent 1000-10,000 MT of associated FM including weed seeds.

Blending: In commercial trading, the quality of grain in demand fluctuates with changing markets and intended uses. Producers, handlers and exporters must balance the costs of cleaning grain against the value it will have on the market. In some cases there may be an incentive for producers or exporters to clean grain to the highest grade or value; however, in many cases there may be market demand for lower quality grain and the incentive is to clean only to the targeted level of the grade or contract

(Johnson and Wilson 1993). To achieve this, many grain elevators use the practice of blending to produce grain with the desired level of FM; that is, rather than cleaning all grain delivered, a portion of high-FM grain is cleaned to a level well below the desired limit and then blended with the rest to achieve the targeted level in the final product (Lin and Lin 1994; Lin 1996). It is unclear to what extent grain lots from different origins are typically blended prior to export, but this could create highly unpredictable weed assemblages in blended grain shipments. The addition of FM back to grain after cleaning is another concern but is prohibited in some countries (e.g., the U.S.) (U.S. Congress Office of Technology Assessment 1989).

Overall, the variation in composition of FM and the practice of blending are significant sources of uncertainty with respect to the potential numbers and species of weed seeds found in grain. Blending of grain lots from different origins with distinct weed floras has the potential to greatly increase the number of weed species in the resultant lot. Unfortunately, information on whether or not a particular grain lot has been blended and the origins of the original grain lots is very difficult, often impossible, to obtain.

Transport and storage

Transport and storage of grain at every stage along the pathway introduces the possibility of cross-contamination and spills. The pathway may be simple or complex in terms of the number of transfers and conveyances prior to arrival at destination. From the point of origin, grain may be moved by truck, rail car and/or ship as it moves towards export and final destination, and may be unloaded and reloaded at a series of intermediate elevators and storage facilities along the way. Each step contributes to uncertainty with respect to the potential for cross-contamination and the risk of spillage post-import.

Cross-contamination: Ideally, good sanitation requires the thorough cleanout of all grain harvesting, transporting, and handling equipment between loads (McNeill and Montross 2003). Practically, however, the cleaning of combines, transportation vehicles and storage facilities between different lots of grain is difficult and often incomplete, resulting in some carryover (Howell and Martens 2002; Shimono and Konuma 2008). The different lots may represent different grades, origins or even crop types. For example, Howell and Martens (2002), report that after careful cleaning of a combine, three bushels of red corn (the original crop harvested) were found in the subsequently harvested yellow corn. In a similar way, weed seed contaminants can get trapped in machinery and end up in subsequent loads of grain.

Accidental spills: Accidental spills are also an unfortunate reality of the grain handling system, as evidenced by the weed and volunteer grain flora along railway tracks, roadsides, ports and around mills and other grain processing facilities (Karnkowski 2001; Dostálek and Jehlík 2004; Jehlík and Dostálek 2008; Hecht et al. 2014; Shimono et al. 2015). Accordingly, roadsides and railways are often included in the habitat

description of ruderal plants (e.g., Darbyshire 2003). In Canada, several occurrences of jointed goatgrass (*Aegilops cylindrica*), a regulated weed, have been reported and subsequently controlled or eradicated along railroad tracks and near port facilities, likely from spills of imported winter wheat (CFIA 2013a). Similarly, in Czechoslovakia, Jehlík and Hejný (1974) documented the main migration routes of adventitious plants into the country with imported grain and agricultural products, showing that many weeds of cereal crops from the U.S.S.R. entered Czechoslovakia following the construction of key railway lines, and subsequently colonized railway stations and warehouses and scattered across the country.

An example of a grain spill on a grand scale is that of a Malaysian cargo ship that went aground in Alaska in 2004, spilling most of a shipment of over 60,000 tons of U.S. No. 2 grade yellow soybeans produced in North Dakota and destined for processing and human consumption in China (Darbyshire and Allison 2009). The soybeans accumulated in large drifts on the shore of Unalaska Island. A 0.25 kg sample of screenings from this shipment was found to contain seeds of more than 46 species of plants, 98% of which were non-native to Unalaska Island, and 85% of which had not previously been reported to occur on the island. Strangely, the sample contained seeds of woolly cup crass (*Eriochloa villosa* (Thunb.) Kunth), which is not naturalized in Alaska or North Dakota. It is unclear how it got into the soybeans, although several possibilities include contamination in transit or in handling facilities, or the blending of soybeans produced in North Dakota with soybeans from states where the species occurs.

As with grain cleaning and blending, the possibility of cross-contamination of conveyances and spills during the transport and storage of grain illustrates the complexity of the pathway and introduces a significant element of uncertainty with respect to the species of weed seeds that might be found in imported grain.

Import requirements

Import requirements are an important means by which countries can reduce the risk of introducing new pests and protect their domestic industries and environments. Currently, all grain imported to Canada is expected to arrive free of soil and regulated pests, and a range of different requirements (e.g., import permits, phytosanitary certificates, treatment certificates) exist for particular crops and countries of origin (CFIA 2015). Pests of concern in imported grain include a number of crop pathogens and stored product pests in addition to weeds (CFIA 2015). Regulated weeds include 20 taxa that have been identified as quarantine (i.e., prohibited) pests under Canada's *Plant Protection Act*, based on pest risk analysis (CFIA 2013b) (Table 2). The absence of regulated pests in imported consignments is typically determined on the basis of area freedom (i.e., pest not present in the area of origin), or where required, certification of official laboratory testing, or acceptable treatment (e.g., heat treatment for devitalization of weed seeds). Non-compliant consignments, when detected, can be prohibited entry or

Table 2. Plants currently regulated as quarantine (i.e., prohibited) pests under Canada's *Plant Protection Act* (CFIA 2013b).

Scientific name	Common name
Aegilops cylindrica Host	Jointed goatgrass
Alopecurus myosuroides Huds.	Slender foxtail
Centaurea iberica Trevir. ex. Spreng.	Iberian starthistle
Centaurea solstitialis L.	Yellow starthistle
Crupina vulgaris Cass.	Common crupina
Cuscuta spp.(except native species)	Dodder
Dioscorea polystachya Turcz.	Chinese yam
Echium plantagineum L.	Paterson's curse
Eriochloa villosa (Thunb.) Kunth	Woolly cup grass
Microstegium vimineum (Trin.) A. Camus	Japanese stiltgrass
Nassella trichotoma (Nees) Hack. ex. Arechav.	Serrated tussock
Orobanche spp. and *Phelipanche* spp. (except native species)	Broomrape
Paspalum dilatatum Poir.	Dallis grass
Persicaria perfoliata (L.) H. Gross	Devil's-tail tearthumb
Pueraria montana (Lour.) Merr.	Kudzu
Senecio inaequidens DC.	South African ragwort
Senecio madagascariensis Poir.	Madagascar ragwort
Solanum elaeagnifolium Cav.	Silverleaf nightshade
Striga spp.	Witchweeds
Zygophyllum fabago L.	Syrian bean-caper

required to be treated. These measures are aimed at reducing the risk of introducing regulated pests and do not necessarily address all pests moving in a pathway.

Inspection sampling data: Compliance with import requirements is monitored through inspection and sampling at the point of import. During the period 2007–2015 an import sampling program focussed on weed seeds in grain was initiated to monitor for regulated species and to gather information about contaminants moving in imported grain. In total, 947 samples were taken from imported shipments of the 10 grain commodities most commonly imported to Canada (see Introduction), and analyzed for presence of weed seeds (Table 3). Sampling was carried out opportunistically by inspectors so the number of samples per crop is uneven (ranging from 7 to 251), making direct comparisons between crops somewhat difficult. However, some broad patterns can still be observed.

Overall, 438 different contaminant taxa were reported in the samples analyzed, including 84 crops present as volunteer weeds or commodity handling contaminants, 288 common weeds already present in Canada, and 66 species which are absent from Canada or very locally introduced (i.e., less than 5 individual locations reported in less than 3 provinces), representing possible new introductions. A number of contaminants were only identified to genus and a few to family; for convenience they are referred to as 'species' from here on. The complete list of contaminants cross-referenced to the

Table 3. Data from a Canadian sampling program showing weed seed contaminant species reported in imported grain 2007–2015. Crop species are provided in Table 1. Contaminant species are separated into: "other crops" (other crop species present as volunteer weeds or commodity handling contaminants); "common weeds" (common weeds and species already established in Canada), and; "new species" (species which are absent from Canada or very locally introduced, representing possible new introductions).

Imported grain	Samples		Range of contaminant species reported per sample	Total number of unique contaminant species reported in all samples			
	Size (kg)	n	(#)	Other Crops (#)	Common Weeds (#)	New species (#)	Total (#)
Corn	1.0	198	0–22	29	74	7	110
Rice	0.5	11	0–12	5	18	4	27
Soybean	1.0	70	0–36	35	99	30	164
Cereals	1.0	223	0–35	55	188	24	267
Pulses	1.0	251	0–36	36	120	4	160
Canola	0.5	52	0–18	18	57	3	78
Sunflower	1.0	42	0–24	22	45	0	67
Flax	0.5	7	0–13	5	21	3	29
Millet	0.5	69	0–18	17	42	3	62
Sorghum	0.5	24	0–16	12	21	1	34
Total		947	0–36	84	288	66	438

crops they were found in is included in Suppl. material 1. All crops sampled contained contaminants, ranging from 27 species in 11 samples (rice) to 267 species in 223 samples (cereals). There was a significant and positive Pearson correlation between the number of samples taken for each crop (n) and the total number of contaminant species reported (correlation = 0.79; p=0.006; n=10), indicating that in general, more sampling is likely to result in more contaminant species reported.

The number of contaminant species per sample ranged from 0 for all crops to between 12 (rice) and 36 (soybean and pulses) (Table 3). Frequency distributions showing the percentage of samples with varying levels of contamination for each crop are included in Suppl. material 2. Rice and soybean had the highest percentage of samples with no contaminants (45.5% and 42.9%, respectively), followed by millet (21.7%), corn (21.2%), sorghum (20.8%) and sunflower (19.0%), while cereals, pulses and canola had the lowest (1.8%, 6.3%, 7.6%, respectively). Patterns for corn and soybean show a relatively high number of samples with no contaminants followed by a steep drop-off, compared with cereals, pulses and canola which have a more even distribution of samples across contaminant levels. Other patterns are less clear (e.g., sorghum, millet, sunflower) or questionable due to limited sample size (e.g., flax, rice). Overall the patterns seem to reflect the relative ease of cleaning large-seeded crops such as corn and soybean compared to those with smaller seeds like cereals and canola. In the case of soybean, the contrast between the large number of samples with no contaminants and the small number of samples with very high numbers of contaminant species (e.g., up to 36 per sample) could be explained by recent trends towards importing organic soybeans; organic grain might be expected to have higher levels of weed seed contam-

Table 4. Top 20 most frequently reported contaminant species in imported grain crops examined in a Canadian sampling program 2007–2015. #Reports (%) indicates the number of samples a species was reported in of a possible 947 with percentages in parentheses, and #Crops indicates the number of crops it was reported in, of a possible 10.

Name of Contaminant	Common name	# Reports (%)	# Crops
Chenopodium album L.	Lamb's-quarters	356 (38%)	10
Fallopia convolvulus (L.) Á. Löve	Wild buckwheat	306 (32 %)	9
Amaranthus retroflexus L.	Redroot pigweed	287 (30%)	9
Setaria italica (L.) P. Beauv. subsp. *viridis* (L.) Thell.	Green foxtail	262 (28 %)	9
Avena fatua L.	Wild oat	241 (25 %)	9
Triticum aestivum L.	Wheat	229 (24 %)	9
Bassia scoparia (L.) A. J. Scott	Kochia	222 (23 %)	9
Thlaspi arvense L.	Stinkweed	198 (21 %)	8
Brassica napus L. subsp. *napus*	Canola or rapeseed	190 (20%)	8
Echinochloa crus-galli (L.) P. Beauv.	Barnyard grass	177 (19%)	10
Sinapis arvensis L.	Wild mustard	143 (15 %)	8
Setaria pumila (Poir.) Roem. & Schult. subsp. *pumila*	Yellow foxtail	127 (13 %)	9
Bromus tectorum L.	Downy brome	122 (13 %)	4
Hordeum vulgare L. subsp. *vulgare*	Barley	111 (12 %)	7
Descurainia sophia (L.) Webb ex Prantl	Flixweed	103 (11 %)	6
Helianthus annuus L.	Sunflower	103 (11 %)	8
Persicaria lapathifolia (L.) Delarbre	Pale smartweed	90 (10%)	10
Salsola tragus L.	Russian thistle	83 (9 %)	8
Cirsium arvense (L.) Scop.	Canada thistle	82 (9 %)	5
Avena sativa L.	Oats	79 (8 %)	8

ination. Pulse samples appear to range fairly evenly across levels of contamination, perhaps because pulses in this case are a mixture of crops of different seed sizes (e.g., beans, peas, chickpeas, lentils). Data for number of contaminants per sample (rather than number of species) were not available at this time.

The 20 most frequently reported contaminant species for all crops combined are shown in Table 4, along with the number of times they were reported and the number of crops they were reported in. All 20 are common crops or weeds in Canada, and not species of phytosanitary concern. Among all 438 contaminant species reported, 58 (13%) were reported in 5 crops or more (>50%), while 241 (55%) were reported in only one crop and 159 (36%) were only reported once (Suppl. material 1). This suggests there is a pool of common weeds moving in multiple crops in the international grain trade, as well as a pool of less common weeds that have specific associations with particular crops or areas of origin. Most "new" contaminant species of phytosanitary concern fall in the latter group. A detailed analysis of contaminant profiles in individual crops would be an interesting area for further study and would support crop-specific risk analyses from different areas of origin. This would allow for comparisons between the weed profiles expected based on field conditions in the country of origin and contaminants found in imported samples. For example, many of the weeds commonly reported in the U.S. corn belt (see Crop-weed associations at the

point of origin, above) are found among the most frequently reported contaminants in imported samples of corn and soybean (e.g., lamb's-quarters, redroot pigweed, green and yellow foxtail, velvetleaf and pigweeds). However, contaminants reported also included less common species, and some surprising associations, e.g., ash (*Fraxinus* spp.) and linden (*Tilia americana* L.) (see Suppl. material 1).

The 66 species that represent potential new weed introductions to Canada are shown in Table 5, along with the number of times they were reported and the number of crops they were reported in. The most frequently encountered were jointed goatgrass (*Aegilops cylindrica*), golden dock (*Rumex maritimus*) and dodder (*Cuscuta* spp.). Jointed goatgrass and dodder are regulated pests under Canada's *Plant Protection Act and Regulations* but are both very difficult to detect and remove from grain, perhaps explaining why they are so frequently reported here. Jointed goatgrass is a crop mimic with seeds that are extremely similar in size and shape to those of wheat and therefore very difficult to clean out of imported wheat commodities (e.g., Chao et al. 2005). Likewise, *Cuscuta* spp. have very small seeds that are difficult to detect and remove, particularly from small-seeded crops (Quasem 2006). The crops with the highest number of "new" species reported were soybean and cereals (Table 3). The list of species in Table 5 could be a useful tool for focussing species-specific pest risk analyses in future.

Overall these results are similar to other studies which have reported large numbers of contaminant weed species in imported grain (Pheloung et al. 1999a; Kurokawa 2001; Shimono and Konuma 2008; Mekky et al. 2010), and indicate that imported grain commodities represent a significant pathway for the introduction of weed seeds regardless of seed size and in spite of cleaning and grading efforts. As with other studies, the contaminants reported here represent a wide range of seed dimensions from very small seeds (e.g., *Amaranthus retroflexus* L. (~1.0 mm) and *Chenopodium album* L. (~1.3 mm)) to larger ones (e.g., *Xanthium strumarium* L. (8–15 mm)) both among and within crops, further suggesting that the effects of grain cleaning on the basis of size, shape and weight is being counteracted along the grain pathway by blending and cross-contamination in transit and storage.

End use of grain in the country of destination

Grain commodities imported to Canada are used for human and animal food as well as industrial products. Wheat, rice, pulses, soybean, canola, sunflower and flax grain are primarily used for human food products in Canada, while corn, barley, oats and sorghum grain are mainly used for livestock feed, and millet grain for bird feed (Small 1999; AERC 2008; ANAC 2012). However, grains are generally multi-purpose and cross over into other usage streams. For example, in addition to its use as animal feed, corn is used for a myriad of human food (e.g., flour, starch, syrup, oil, hominy, grits) and industrial products (e.g., plastics, fabrics, ethanol). Similarly, significant amounts of barley are used in the malting industry. Interestingly, almost any type of grain can end up in the animal

Table 5. Contaminants that represent potential new weed species introductions to Canada, reported in imported grain crops examined in a Canadian sampling program 2007–2015. #Reports indicates the number of samples a species was reported in of a possible 947, and #Crops indicates the number of crops it was reported in, of a possible 10.

Name of contaminant	#Reports	# Crops	Name of contaminant	#Reports	# Crops
Aegilops cylindrica Host	54	2	*Anchusa azurea* Mill.	1	1
Rumex maritimus L.	22	3	*Anoda* spp.	1	1
Cuscuta spp.	10	4	*Blainvillea acmella* (L.) Philipson	1	1
Commelina benghalensis L.	7	1	*Bromus sterilis* L.	1	1
Digera muricata (L.) Mart.	5	1	*Codonopsis* spp.	1	1
Phaseolus spp. (except crops)	5	1	*Crambe* spp.	1	1
Rapistrum rugosum (L.) All.	5	1	*Cyanotis axillaris* (L.) D. Don	1	1
Euphorbia heterophylla L.	4	1	*Cynodon dactylon* (L.) Pers.	1	1
Apera spica-venti (L.) P. Beauv.	3	1	*Dactyloctenium aegyptium* (L.) Willd.	1	1
Consolida regalis Gray	3	1	*Gaillardia megapotamica* (Spreng.) Baker	1	1
Digitaria ciliaris (Retz.) Koeler	3	2	*Galium tricornutum* Dandy	1	1
Dinebra retroflexa (Vahl) Panz.	3	1	*Ipomoea hederacea* Jacq.	1	1
Eleusine indica (L.) Gaertn.	3	1	*Ipomoea lacunosa* L.	1	1
Hirschfeldia incana (L.) Lagr.-Foss.	3	1	*Lepyrodiclis holosteoides* (C. A. Mey.) Fenzl ex Fisch. & C. A. Mey.	1	1
Alisma plantago-aquatica L.	2	2	*Pedaliaceae* spp.	1	1
Bromus arvensis L.	2	1	*Pennisetum* spp.	1	1
Bromus catharticus Vahl var. *catharticus*	2	1	*Perilla frutescens* (L.) Britton	1	1
Celosia argentea L.	2	2	*Persicaria nepalensis* (Meisn.) H. Gross	1	1
Corchorus olitorius L.	2	1	*Phyllanthus* spp.	1	1
Cucumis spp. (except crops)	2	1	*Rapistrum perenne* (L.) All.	1	1
Euphorbia davidii Subils	2	1	*Rapistrum* spp.	1	1
Glaucium corniculatum (L.) Rudolph	2	1	*Reseda odorata* L.	1	1
Nicandra physalodes (L.) Gaertn.	2	2	*Rorippa islandica* (Oeder) Borbás	1	1
Panicum psilopodium Trin.	2	2	*Salvia hispanica* L.		1
Phyllanthus urinaria L.	2	1	*Sesbania exaltata* (Raf.) Rydb.	1	1
Rottboellia cochinchinensis (Lour.) Clayton	2	1	*Setaria pumila* (Poir.) Roem. & Schult. subsp. *subtesselata* (Büse) B. K. Simon	1	1
Salvia columbariae Benth.	2	2	*Sida spinosa* L.	1	1
Schoenoplectiella mucronata (L.) J. Jung & H. K. Choi	2	1	*Sisymbrium orientale* L.	1	1
Sida rhombifolia L.	2	2	*Spermacoce* spp.	1	1
Urochloa fusca (Sw.) B. F. Hansen & Wunderlin	2	2	*Stachys annua* (L.) L.	1	1
Achyranthes aspera L.	1	1	*Trifolium reflexum* L.	1	1
Alternanthera ficoidea (L.) P. Beauv.	1	1	*Verbena officinalis* L.	1	1
Amaranthus caudatus L.	1	1	*Veronica hederifolia* L.	1	1

feed stream, either in whole or by-product form. Distillers' grains, a by-product of corn ethanol production (Heuzé et al. 2015) and canola meal, a by-product of canola oil production, are just two examples among many (Casséus 2009).

Human and industrial uses: Grain for human consumption or industrial uses is typically cleaned to a very high standard. Beyond the cleaning undertaken to meet grade or contract specifications prior to export, imported grain for human food or industrial end uses typically undergoes further cleaning in order to ensure quality and consistency of the resultant products (Matz 1991; Catania et al. 1992; Delcour and Hoseney 2010). The by-product of any cleaning process is screenings, discussed separately in the next section. Grain processing for food or industrial products may be partially to totally destructive, and can include decortication, polishing, milling, extraction, malting, fermentation, cooking, parboiling, and other commercial processes (Delcour and Hoseney 2010). Many of the commodities resulting from these processes are categorized according their level of pest risk in an international standard produced by the IPPC (ISPM 32) (IPPC 2009). In general, the initial grain cleaning in conjunction with these destructive mechanical, chemical and thermal treatments seems almost certain to reduce the number of contaminating viable weed seeds in the ensuing products and by-products to negligible levels, thereby mitigating any significant risk for the introduction of weed seeds. Direct evidence for this is lacking and further research into the effects of these processes on weed seed viability would be useful to clarify the relative level of risk.

In Canada, many imported grain commodities are used as livestock feed (AAFC 2009; AAFC 2010; Gabruch and Gietz 2014). Compared with grain used for human food or industrial processes (and their by-products), grain used for animal feed may be cleaned and processed to lesser degrees (CGC 2015). With some exceptions, most grains can be fed whole, although they are more often ground or rolled to improve the feed value and digestibility (Marx et al. 2000). Livestock feed that undergoes minimal or no processing is of particular concern, as it may contain weed seeds that can be subsequently spilled on the ground or pass through the digestive tracts of animals while retaining their viability (Blackshaw and Rode 1991; Kurokawa 2001). The most important livestock feeds in Canada are barley grain in the west and corn grain in the east (Small 1999). Feed peas, wheat, oats, and canola and soybean meal are also important inputs in Canadian livestock feeds (Small 1999; Hickling 2003; Newkirk 2010). The other grains covered in this document, including flax, millet, rice, sorghum, sunflower, and pulses other than feed peas, are only of minor importance for use in livestock feed in Canada. However, it should be noted that millet, sunflower and sorghum grain used for bird feed are unlikely to undergo any processing at all.

Livestock feed that is processed can undergo a number of transformative processes including particle size reduction by grinding or rolling with a hammer or roller mill, conditioning, pelleting and extrusion (Guyer 1973; Canadian Feed Industry Association 1984). Particle size reduction processing significantly reduces, but does not eliminate the viability of contaminating weed seeds in grain (Zamora and Olivarez 1994). Conditioning refers to the addition of moisture to bring the grain to an optimum level for processing, usually in the presence of heat (82–100 °C) (Canadian Feed Industry Association 1984). Pelleting and extrusion are similar processes in which feed mixtures are forced through the holes of a die plate, and also involve the generation of or ex-

posure to heat (about 80 °C for pelleting and up to 200 °C for extrusion) (Lević and Sredanović 2010). Pelleting or extrusion in combination with particle size reduction has been shown to be more effective at reducing the viability of contaminating weed seeds than particle size reduction alone (Cash et al. 1998; Zamora and Olivarez 1994). Zamora and Olivarez (1994) tested the viability of alfalfa seeds after grinding and/or formation of grain pellets using steam. From an original viability of 94%, ground unpelleted alfalfa seeds were still 91.5% viable whereas ground and pelleted seed were 52.5% viable. In a study by Cash et al. (1998), only very small quantities of alfalfa seed (0.01–0.50%) germinated after typical commercial feed manufacturing processes, which included grinding and pelleting. Each step in the feed processing sequence resulted in fewer viable seeds, with the majority of seed mortality being attributed to grinding and the adjustment of settings to achieve smaller particle size.

End use processing can clearly mitigate the risk of weed seed introduction in many cases, and is an important consideration in a pathway risk analysis for imported grain. Grain subject to cleaning and processing for human consumption and industrial uses presents a low risk of introducing weeds into new environments, as weed seeds are either removed during cleaning or devitalized during processing. In contrast, livestock and bird feeds subject to minimal processing represent a higher risk for the transmission of viable weed seeds. It is expected that the greater the degree of processing, the less likely the feed will contain viable weed seeds.

Screenings as a by-product of grain cleaning: Grain screenings represent a high risk relative to the grain they originate from, because they represent a concentration of the non-grain fraction that includes weed seeds and other material that remains after the grain has been cleaned. In Canada, grain screenings are most frequently used as components in livestock feed. The raw screenings are often processed by grinding and pelleting to reduce problems with feeding and handling. One study in Saskatchewan indicated that weed seed viability was almost completely destroyed in grain screenings that had been ground and steam pelleted and/or treated with ammonia (Janzen 1995). Likewise, the Canadian Food Inspection Agency (CFIA) monitors domestic grain screening pellets exported to the U.S. to ensure they meet phytosanitary requirements and has shown that grinding and heating during pelletization renders weed seeds non-viable (CFIA 2012; CFIA Saskatoon Laboratory Seed Science and Technology Section, pers. comm.).

However, screenings that are unprocessed or ground but not further processed present a potential risk for the introduction of weed seeds to farm properties and elsewhere. Studies have shown that sheep and steers fed unprocessed grain screenings had viable weed seeds in their manure (Janzen 1995). Similarly, Scott et al. (1950) found refuse screenings that had been ground on a 3/8 inch screen contained several contaminant species, with amounts varying from 453 seeds per pound of screenings (wild oats (*Avena fatua* L.), mustard (*Brassica* spp.), fiddleneck (*Amsinckia* spp.)) to 44,492 seeds per pound (lamb's-quarters (*Chenopodium album* L.)). In another study, samples of screenings were collected from eleven grain elevators in Saskatchewan, separated into fine, medium and coarse particle-size fractions, and processed through various settings

on hammer-mills and roller mills and then tested for seed germination. The results showed that the effectiveness of hammer and roller mills for destroying weed seeds increased with decreased screen mesh size and roller spacing, respectively. However, none of the treatments were 100% effective at destroying weed seeds in the fine fractions, which would have contained the tiniest weed seeds (AFMRC and PAMI 2000).

Of all the end uses of grains, unprocessed or minimally processed screenings present the highest risk for containing viable weed seeds, and potentially large numbers of them. The weeds seeds in screenings can be unintentionally spilled in a variety of environments conducive to germination, including areas around mills, bins and farm properties, or be fed to livestock and dispersed into pastures. To address the risk posed by imported, unprocessed screenings and grain for cleaning (which generates screenings), import requirements have been established in Canada (CFIA 2013c), requiring the material to be transported in such a way as to avoid spillage or spread, and cleaned (in the case of grain) or pelleted or milled (for screenings) as soon as possible after entry. Furthermore, residual materials must be securely contained and disposed of, such as by burning or burial. This suggests that much of the risk posed by imported screenings and grain for cleaning has been mitigated through regulation, however, it is unclear to what extent this applies to imported grain designated for other end uses.

Conclusions

In summary, imported grains represent a very complex pathway for the possible introduction of new weed species to Canada. Weed-crop associations at the point of origin, along with crop production and harvesting practices, can be researched to develop predictions of what weed species might be associated with which imports; however, subsequent steps along the pathway such as grain cleaning, blending, and the potential for cross-contamination in transport and storage mean the weeds found in import sampling programs are not always the ones that might be expected. Import interception data presented here shows that all imported grain commodities sampled were a source of associated weed contaminants, however information about end use indicates that grain destined for human food or industrial purposes in Canada likely presents a negligible risk of introducing new weeds into the environment, due to extensive cleaning and processing at destination. Further research on the effects of specific processes on weed seed viability would be useful to confirm this. However, the greater risk lies with imported grain that is direct-fed or minimally processed for livestock feed, and the fate of dockage or screenings that are removed from grain during the cleaning process.

The pathway risk analysis approach provides a useful framework for characterizing the nature of a pathway, identifying events that affect pest risk, and highlighting possibilities for risk reduction or mitigation. In this case, a qualitative description of the pathway from point of origin to end use at destination provides a better understanding of the multiple interacting factors that may affect weed seed contamination in grain imports, and this may help to focus plant protection efforts in future. For example,

future risk analyses on specific grain commodities may call for less focus on the analysis of crop-weed associations at the point of origin and production and harvesting practices and more focus on end use. Likewise, risk mitigation efforts might be most usefully focused on grain used for livestock feed and management of screenings, as compared to grain for human consumption or industrial purposes which present little risk of introducing new weeds to the environment.

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Supplementary material 1

Weed seed contaminant species reported in imported grain in a Canadian sampling program 2007–2015

Authors: Claire E. Wilson, Karen L. Castro, Graham B. Thurston, Andrea Sissons

Data type: Species list and tabular occurrence data

Explanation note: Complete list of weed seed contaminant species reported in 947 samples of 10 imported grain crops in a Canadian sampling program 2007–2015, cross-listed to number of times reported and crops reported in.

Supplementary material 2

Frequency distributions showing percentage samples with number of contaminant species reported per sample for 10 imported grain crops examined in a Canadian sampling program 2007–2015

Authors: Claire E. Wilson, Karen L. Castro, Graham B. Thurston, Andrea Sissons

Data type: Frequency distribution graphs

Explanation note: Ten frequency distribution graphs (one per crop) shown in a multi-panel.